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THE RELATIONSHIP OF A LOW BLOOD CALCIUM TO PARATHYROID SECRETION

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
1942

By
HARVEY M. PATT

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RELATIONSHIP OF A LOW BLOOD CALCIUM TO PARATHYROID SECRETION

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WHETHER the parathyroid glands are subject to nervous control is not known. L. R. Dragstedt (personal communication) stimulated the cervical sympathetic for hours and was unable to detect any change in the blood calcium and phosphorus. Transplantation experiments have demonstrated that the parathyroid glands can function adequately in the absence of all nervous connections (1). The evidence would seem to indicate that the nerves have vasomotor rather than secretory functions.

There is some evidence that the parathyroid glands may be under the influence of a hormone from the anterior lobe of the hypophysis. This principle has been variously called parathyrotropin and parathyrostimulin. The association in the human subject of parathyroid adenomas with tumors of the hypophysis has been reported (2). Houssay and Sammartino (3) observed degenerative lesions of the parathyroid in 66 per cent of all hypophysectomized dogs. Smith (4) reported atrophy of the parathyroids following pituitary ablation in the rat. However, Aschner (5) was unable to detect any change in the parathyroid glands of dogs following hypophysectomy. A few investigators have reported parathyroid enlargement and other structural changes following the injection of anterior pituitary extract (3). Although the evidence would seem to indicate a pituitary-parathyroid relationship, the existence of a specific parathyrotropic principle has not yet been proved.

It is possible that one factor in the control of the parathyroid glands is a local response of the gland cells to chemical changes in the composition of the surrounding fluids. There is considerable circumstantial evidence that a low serum calcium is a stimulus for the parathyroid glands to produce more hormone. Parathyroid hyperplasia may be induced experimentally by low calcium diets (6). The recent investigations by Sinclair (7) indicate that fetal rat parathyroids are stimulated by a subnormal maternal serum calcium or an excess of maternal serum phosphorus. The criterion for stimulation was an increased size of the gland at term. The fetal parathyroid glands were also shown to be depressed by an excess of parathyroid hormone from an enlarged maternal gland or by an excess of maternal serum calcium.

Clinically, hyperplasia of parathyroid tissue may occur in association with chronic nephritis (8). This may be a compensatory reaction to the hypocalcemia which is the result of a phosphate retention and consequent hyperphosphatemia. Albright (9) believes that there may be a hyperfunction of the parathyroid glands in vitamin D insufficiency (rickets and osteomalacia), the stimulus being the low level of serum calcium resulting from the interference with the absorption of calcium from the gastrointestinal tract. The increased production of parathyroid hormone leads to a decrease in serum inorganic phosphorus but maintains the serum calcium at its normal level.

According to Helfet (10) an accumulation of phosphate in the blood is a stimulus

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to increased secretion of parathyroid hormone. However, it must be remembered that there is a reciprocal relationship between the blood calcium and phosphorus such that if phosphorus is elevated there is usually a decrease in calcium. Thus the indirect evidence presented by Helfet in favor of a high blood phosphorus stimulation of the parathyroids could hold equally well for the low blood calcium idea of stimulation.

In a preliminary report Patt, Wallerstein and Luckhardt (11) presented evidence for a humoral control of parathyroid secretion by the blood calcium. It is the purpose of the present paper to present a more detailed account as well as an extension of the above investigation.

Experimental Hypocalcemia

METHODS

Transfusion with decalcified blood. For the production of a hypocalcemia the method of Hastings and Huggins was used (12). Several large dogs were bled maximally and the blood defibrinated and centrifuged. The serum was decalcified by shaking with solid lead phosphate and then centrifuged to remove the lead phosphate with the adsorbed calcium. The solid lead phosphate adsorbs about 80 to 90 per cent of the calcium. The serum was subsequently remixed with the blood cells to yield decalcified blood.

Small dogs (anesthetized with nembutal or with ether) were alternately bled maximally and immediately transfused with an equal volume of decalcified blood. This procedure was repeated every 10 minutes for 2 to 2.5 hours in both normal dogs and dogs thyroparathyroidectomized just prior to the onset of the bleedings and transfusions. Blood samples were taken during the course of the experiment. Serum calcium was determined by the method of Kramer and Tisdall (13) as modified by Clark and Collip (14). In several experiments the total protein concentration was obtained by the micro-Kjeldahl method of Campbell and Hanna (15). The distillation of the ammonia was carried out in the Goebel modification of the Pregl micro-Kjeldahl distillation apparatus (16). The proteins were estimated by multiplying the total nitrogen corrected for non-protein nitrogen by 6.25. When protein determinations were made the ionic calcium was calculated using the nomogram of McLean and Hastings (17).

Injection of sodium oxalate. Dogs were anesthetized by an intravenous injection of sodium barbital (250-70 mg./kg.). Large amounts of sodium oxalate (40 mg./kg.) were injected intravenously both into normal dogs and into dogs thyroparathyroidectomized just prior to the oxalate injections. It was found that the animals would tolerate the massive amounts of sodium oxalate if the latter was dissolved in 50 to 100 cc. of 0.9 per cent NaCl and the total dose injected over a period of 30-45 minutes.

RESULTS AND DISCUSSION

Confirming the findings of Hastings and Huggins (12), the time interval between transfusions was found to be of great importance in lowering the serum calcium to tetany levels; that is, at 20-minute intervals or higher, the calcium did not drop appreciably, but at 10-minute intervals the total serum calcium decreased to 5.5-7 mg. per cent after about 14 transfusions, and in several dogs localized muscle fibrillations and tremors were seen.

Twenty to 30 minutes after the last transfusion the total serum calcium rose 1 to 2 mg. both in the normal dogs and in dogs thyroparathyroidectomized just prior to the experiment. Subsequent samples, 40 and 80 minutes after the last transfusion, showed no further rise in serum calcium. The results obtained on 7 normal and 3 parathyroprival dogs are indicated in table 1.

TABLE 1. CHANGES IN SERUM CALCIUM, TOTAL PROTEIN, AND IONIC CALCIUM FOLLOWING ALTERNATE BLEEDING AND TRANSFUSION WITH DECALCIFIED BLOOD

		Normal Dogs							Parathyroprival Dogs		
		1	2	3	4	5	6	7	8	9	10
Normal Blood	Total serum Ca., mg. %	9.98	10.8	9.31	9.88	10.4	10.3	9.45	11.7	9.45	10.7
	Total protein, gm. %				5.76	5.98	6.96	5.65			4.45
	Ionic Ca., mg. %				4.8	5.0	4.6	4.8			6.0
After 15 Transfusions	Total serum Ca., mg. %	6.95	7.2	5.78	6.24	5.78	6.66	5.79	6.89	5.46	6.51
	Total protein, gm. %				8.75	4.64	4.58	5.92			6.75
	Ionic Ca., mg. %				2.4	3.0	3.6	2.7			2.8
1 hour after 15th Transfusion	Total serum Ca., mg. %	8.82	7.8	7.46	7.6	7.35	7.38	7.35	8.16	7.8	8.4
	Total protein, gm. %				8.54	4.48	4.82	6.1			7.54
	Ionic Ca., mg. %				2.8	4.1	3.9	3.5			3.5

Since the rise in calcium was of the same magnitude and occurred at approximately the same time in both the normal and the thyroparathyroidectomized dogs, it is clear that a mechanism other than parathyroid activity must be responsible. Possible sources of this calcium are, a) the tissue fluids, b), the bone salts.

Owing to the permeability of the capillaries to the diffusible calcium, the calcium in the blood is in equilibrium with the calcium of the tissue fluids. Should the diffusible calcium of the blood be lowered, a diffusion gradient would be set up, calcium diffusing from the tissues to re-establish the equilibrium. However, according to Hastings and Huggins (12) the calcium removed by the above procedures had exceeded that calculated as being initially present in solution in the body. They interpret this as evidence that the bone salts are freely soluble in serum which has been rendered undersaturated with respect to the bone salts.

In the light of other experiments involving the intravenous injection of sodium oxalate, it is quite probable that had these experiments been continued longer there

TABLE 2. SERUM CALCIUM FOLLOWING INTRAVENOUS INJECTION OF SODIUM OXALATE (40 mg./kg.)

Dog	Serum Calcium, mg. %		
	Normal blood	30 minutes after oxalate injection	5-6 hours after oxalate injection
1, Normal	11.2	6.03	9.88
2, Normal	9.6	7.1	10.08
3, Normal	11.75	5.41	8.84
4, Normal	10.5	6.76	9.46
5, Parathyroprival	9.36	6.34	7.12
6, Parathyroprival	9.6	5.71	7.06
7, Normal	10.5	5.65	9.98
8, Parathyroprival	10.4	5.72	7.64
9, Normal	9.2	3.45	6.95
10, Normal	9.17	2.45	7.12

would have been a distinct difference in the blood calcium level of the normal and parathyroprival dogs.

The changes in serum calcium following the intravenous injection of sodium oxalate are shown in table 2. The serum calcium fell immediately after the oxalate injections, but rose promptly in the normal dogs, and within 1.5 to 3.5 hours returned to the normal level in 3 of the animals. In 4 of the normal dogs there was an increase in serum calcium of 3.85, 3.43, 3.5, and 4.67 mg. A rise in serum calcium of 0.78, 1.35, and 1.92 mg. occurred in the parathyroprival dogs and there was no further change although the observations extended over a 7-hour period (fig. 1, 2).

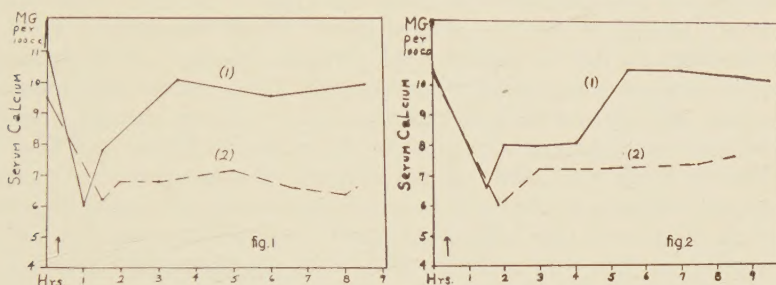


Fig. 1, 2. SERUM CALCIUM FOLLOWING INTRAVENOUS INJECTION of sodium oxalate into (1) normal dog and (2) dog thyroparathyroidectomized just prior to oxalate injection.

In several animals irregular respiratory movements followed by cardiac arrest in diastole occurred during the course of the oxalate injections. This was attributed to the precipitous fall in blood calcium induced by a too-rapid injection of a concentrated oxalate solution. Goodman and Gilman (18) state that the main pharmacological action of sodium oxalate is due to its ability to precipitate calcium from the blood and body fluids.

The fact that only the dogs with intact parathyroids possessed the ability to restore their blood calcium to normal following the intravenous injection of sodium oxalate (where there was no complete return to normal, the rise was at least twice that observed in the parathyroprival animals) indicates that the parathyroids are a necessary part of the restorative mechanism. Whether the low blood calcium is a direct stimulus to the parathyroid glands or acts indirectly, perhaps by way of the pituitary or some other intermediary, is not evident from these experiments.

The small rise in serum calcium occurring in the thyroparathyroidectomized animals is almost identical with the results obtained in similar animals in the first series of experiments. The calcium in this instance may possibly come from the tissue fluids, the bone salts, or perhaps there is a dissolution of part of the calcium oxalate precipitate by the reticulo-endothelial system. More likely there is an excretion of oxalate, some of the calcium thus being freed.

Thyroid-Parathyroid Perfusion

METHODS

A more direct experiment was performed as follows. A large dog was anesthetized by an intravenous injection of sodium barbital (270 mg./kg.). The femoral artery was cannulated and 400 to 500 cc. of blood collected. Heparin was added as

an anticoagulant. The blood was decalcified with phosphate in the manner described above.

The thyroid-parathyroid gland was isolated from the systemic circulation by tying appropriate blood vessels, leaving open only one artery (the superior thyroid branch of the carotid) and one vein (the internal jugular into which several thyroid veins empty). The carotid artery was ligated above and below the origin of the superior thyroid artery and a cannula inserted between the ligatures. The internal jugular vein was also cannulated. Decalcified heparinized blood was perfused through the gland using the ordinary drip method (that is, by gravity) for 1.5 to 2 hours at a rate of approximately 3 to 4 cc. per minute, and the blood coming from the thyroid and parathyroids was collected. The animal was killed at the onset of the perfusion to insure the passage of only the perfused blood through the prepared gland.

On the following day the plasma of this perfusate (50-75 cc. of decalcified blood parathyroid perfusate) was injected intravenously into a normal dog (anesthetized with sodium barbital) and the serum calcium and phosphorus changes were noted. Serum calcium determinations were made by the method of Kramer and Tisdall (13) as modified by Clark and Collip (14) and inorganic phosphorus was determined by the Fiske and Subbarow method (19). In several experiments the dogs were given 400 cc. of water by stomach tube before injection of the perfusate to insure a fairly constant diuresis and then were catheterized. Urinary phosphorus was determined by the method of Fiske and Subbarow (19).

In the control experiments the same procedures were adhered to but normal heparinized blood was perfused through the gland. The collected perfusate (normal blood parathyroid perfusate) was injected as described above. In two experiments simultaneous urine samples were taken and the values for urinary phosphorus determined.

Histologic studies were made in several experiments using the unperfused gland as a control for the gland perfused with decalcified or normal blood. The thyroid-parathyroid glands were fixed in Zenker-formol and after sectioning were stained with hematoxylin-eosin-azure.

RESULTS AND DISCUSSION

The maximum changes in serum calcium and serum inorganic phosphorus following the intravenous injection of the decalcified blood parathyroid perfusate are indicated in table 3. The average increase in serum calcium was 2.22 mg. and in inorganic phosphorus 2.31 mg.

TABLE 3. MAXIMUM CHANGES IN SERUM CALCIUM AND SERUM INORGANIC PHOSPHORUS FOLLOWING INJECTION OF DECALCIFIED BLOOD PARATHYROID PERFUSATE

Dog	Serum, Calcium, mg. %	Serum Inorg. Phosphorus, mg. %	Dog	Serum Calcium, mg. %	Serum Inorg. Phosphorus, mg. %
1	2.71	2.68	8 ²	3.48	3.38
2	4.94	5.54	9	1.33	1.40
3	1.6		10	3.2	
4 ¹	1.95	-1.98	11	1.53	2.38
5	2.9	2.27	12	0.94	
6	2.29	1.59	13	1.94	-0.76
7	0.82		14	0.51	
Average, excluding dog 4,				2.22	2.31

¹ Chronic parathyroidectomized dog.

² Ureters ligated.

In 11 dogs there was a rise in serum calcium of from 1.3 to 4.9 mg. within 1.5 to 3 hours after the injection (fig. 3-7). Three animals (7, 12, 14 in table 3) showed an elevation in serum calcium of only 0.82, 0.94, and 0.51 mg. The failure of the latter to give a definite increase is not altogether clear. In dog 14 the vein draining the inferior pole of the thyroid was destroyed during the dissection. It is possible that the latter is the most important venous channel for the parathyroid glands. However, the presence of extensive venous anastomoses in the thyroid might not support this contention. Dogs 7 and 12 were studied for only 5 hours after the injection, and in a

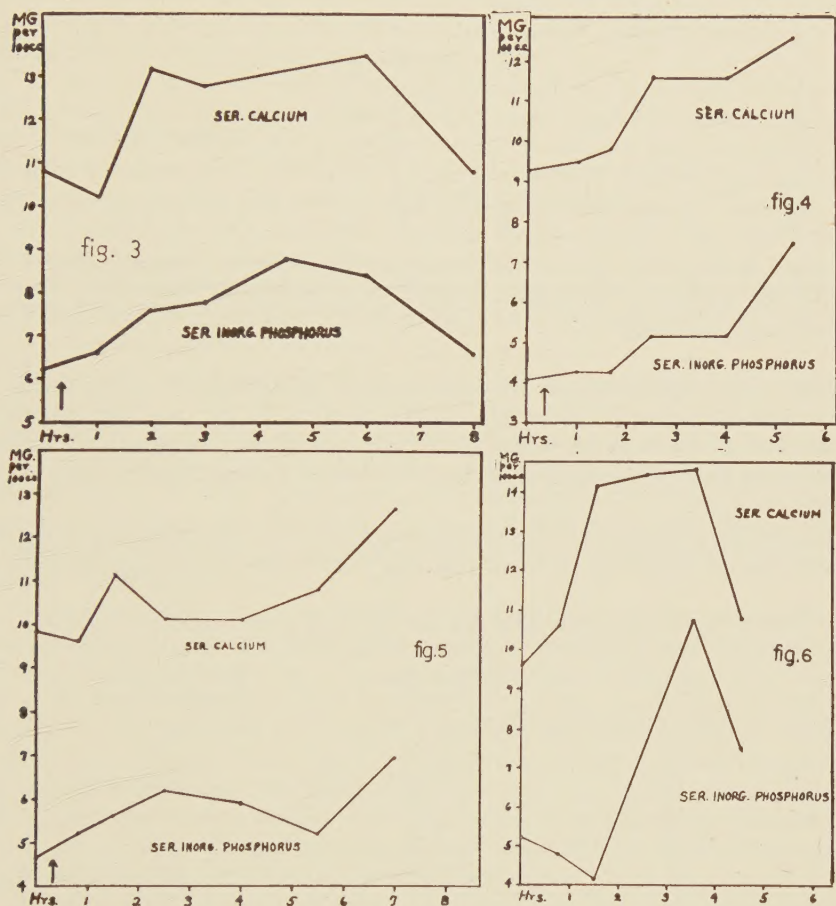


Fig. 3-6. SERUM CALCIUM AND SERUM INORGANIC PHOSPHORUS following intravenous injection of decalcified blood parathyroid perfusate into normal dogs.

previous experiment the maximum rise in calcium occurred after 7 hours. There was also an increase in serum inorganic phosphorus in 7 dogs, but the maximum elevation in phosphorus occurred somewhat after that of calcium. In dog 2 the rise in phosphorus was preceded by a fall, while in dog 13 there was a fall followed only by a return to the normal level. Dog 4 (chronic parathyropival) showed a fall in serum inorganic phosphorus. Urine samples were collected in dogs 10, 11, and 13. There was an increase in phosphate excretion within 1 to 2 hours after the injection. The maximum increase in urinary phosphorus was 74.4, 46.4, and 25.5 mg. per cent.

The normal blood parathyroid perfusate upon intravenous injection yielded no significant change in serum calcium, the maximum being 0.66 mg. (table 4; fig. 8). The serum inorganic phosphorus was followed in 4 of the dogs, 2 of which showed no change. In dog 17 there was an increase of 1.54 mg. but this may be due to phosphate retention by the kidneys since the ureters were ligated. Dog 21 was of especial

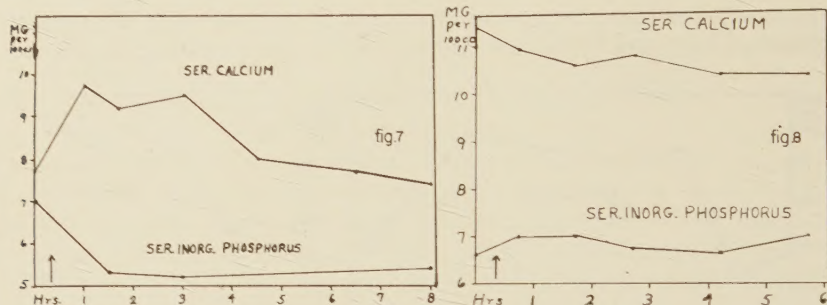


Fig. 7. SERUM CALCIUM AND SERUM INORGANIC PHOSPHORUS following intravenous injection of decalcified blood parathyroid perfusate into a dog parathyroidectomized 5 days before. Fig. 8. SERUM CALCIUM AND SERUM INORGANIC PHOSPHORUS following intravenous injection of normal blood parathyroid perfusate into a normal dog.

interest. There was an increase in serum inorganic phosphorus of 3.3 mg. and at the same time a decrease in urinary phosphorus excretion of 17.7 mg. per cent. Here, apparently, the rise in serum phosphorus is due to phosphate retention by the kidneys. In the next hour the serum and urinary phosphorus returned toward their normal levels.

According to Logan (20) the urinary phosphorus excretion of fasting young dogs is not constant from hour to hour. This may account for the changes in phosphate

TABLE 4. BLOOD CHANGES FOLLOWING INJECTION OF NORMAL BLOOD PARATHYROID PERFUSATE

Dog	Serum Calcium, mg. %	Serum Inorganic Phosphorus, mg.
15	-0.8	0.45
16	-0.57	
17 ¹	0.51	1.54
18	0.66	
19	0.52	
20	0.16	-0.1
21	-0.60	3.3

¹ Ureters ligated.

excretion observed after injection of the normal blood parathyroid perfusate as well as after the decalcified blood parathyroid perfusate. However, in the two animals observed after injection of the normal blood parathyroid perfusate, the change was mainly in the direction of a decrease, but our experimental studies on phosphate excretion following the injection of both types of perfusate are too few to be significant.

Histologic studies failed to demonstrate any significant difference between the parathyroids perfused with normal or decalcified blood as compared with the control unperfused gland. Sections were taken of 5 control and 5 perfused glands, 2 of the

latter having been perfused with normal blood and 3 with decalcified blood. In all sections the epithelial cells were normal in appearance. In 2 glands perfused with decalcified blood and in one gland perfused with normal blood, crenated red cells and some bacteria were detected in several of the blood vessels. However, the presence of bacteria is not surprising, since no aseptic precautions were taken during the perfusion. Cytological studies (for example, osmic acid impregnation) might demonstrate a significant difference between the glands perfused with normal and decalcified blood.

Since comparatively little is known of the chemistry of the parathyroid hormone and there is no chemical test available for its detection, a biological method (rise in blood calcium) was used to determine its presence.

The fact that there was a significant elevation in serum calcium when decalcified blood was perfused through the thyroid-parathyroid apparatus and the perfusate injected into a dog indicates the presence of an active calcium-raising principle in the perfusate. Whether this is identical with the hormone as secreted physiologically by the parathyroid cells will have to await chemical identification. The changes in the serum phosphorus are not in agreement with the results usually obtained following the administration of parathyroid extract. Logan (20) reported a decrease in inorganic phosphorus as well as an increase in serum calcium during the first or second hour following the intravenous injection of parathyroid extract. There was also an increase in phosphate excretion at this time.

The apparent discrepancy in the direction of serum phosphorus changes may be due, conceivably, to differences in the concentration of 'hormone' used in the two experiments. It would seem that the perfusate contains a high parathyroid hormone titer. If the active principle in the perfusate is acting on the bone salt to liberate calcium, the increase in serum phosphorus is not unreasonable. The direction of change in serum phosphorus is probably dependent upon the ratio of the increased amount of phosphorus liberated from the bone salt to the increased amount of phosphorus excreted by the kidneys.

Another possibility must be considered. Is parathyroid extract identical with the hormone secreted by the parathyroid gland into the blood? Likewise, is the calcium-raising principle in the perfusate this same hormone or perhaps only an active part of the so-called parathormone? Sinclair (7) found that the function of fetal rat parathyroids was depressed by an excess of parathyroid hormone from an enlarged maternal gland. In order to affect the fetal parathyroids the hormone must necessarily pass through the placenta. Sinclair suggests that since parathyroid extract is a colloidal protein and there is some evidence that molecules of this size do not pass the placenta, it would appear that the extract is not identical with the secretion of the parathyroid gland as it is liberated into the blood stream.

The experiments reported are interpreted as evidence that a low blood calcium is a direct stimulus for the parathyroid glands to produce more hormone, thus indicating a humoral control of parathyroid secretion.

SUMMARY

1. After alternate bleeding and transfusion of decalcified blood into both normal and acutely thyroparathyroidectomized dogs there was a fall in serum calcium to 5.5-7 mg. per cent. Twenty to 30 minutes after the last transfusion the serum calcium rose 1 to 2 mg. in both groups and no further change was observed during the next hour. This negative finding might be due to the short duration of the experiments.
2. After the intravenous injection of sodium oxalate (40 mg. per kg.) into normal

and acutely thyroparathyroidectomized dogs, there was a fall in serum calcium to 7 mg. per cent or below, followed by a return to normal (within 1.5 to 3.5 hours) only in the intact animals. When there was no complete return to normal, the rise in serum calcium was at least twice that observed in the parathyroidectomized animals.

3. When decalcified blood was perfused through the thyroid parathyroid preparation and the perfusate injected intravenously into normal dogs there was an increase in serum calcium of 1.3 to 4.9 mg. within 1.5 to 3 hours, and in most instances an increase in serum inorganic phosphorus of almost the same magnitude.

4. However, when normal blood was perfused through the thyroid parathyroid gland, the collected perfusate upon intravenous injection yielded no significant change in serum calcium.

5. Histologic studies using a hematoxylin eosin azure stain failed to demonstrate any significant difference between parathyroid gland tissue perfused with normal or decalcified blood as compared with the control unperfused gland. After perfusion the epithelial cells were normal in appearance.

6. The experiments reported are interpreted as evidence that a low blood calcium is a direct stimulus for the parathyroid glands to produce more hormone, thus indicating a humoral control of parathyroid secretion.

The author wishes to express his sincere gratitude to Dr. Arno B. Lockhardt for suggesting the problem and for his valuable advice and guidance.

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